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Journal

The Journal of cell biology, 167(4)

ISSN

0021-9525

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Publication Date

2004-11-01

DOI

10.1083/jcb.200408061

Peer reviewed

Condensin restructures chromosomes in preparation for meiotic divisions

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The production of haploid gametes from diploid germ cells requires two rounds of meiotic chromosome segregation after one round of replication. Accurate meiotic chromosome segregation involves the remodeling of each pair of homologous chromosomes around the site of crossover into a highly condensed and ordered structure. We showed that condensin, the protein complex needed for mitotic chromosome compaction, restructures chromosomes during meiosis in *Caenorhabditis*

elegans. In particular, condensin promotes both meiotic chromosome condensation after crossover recombination and the remodeling of sister chromatids. Condensin helps resolve cohesin-independent linkages between sister chromatids and alleviates recombination-independent linkages between homologues. The safeguarding of chromosome resolution by condensin permits chromosome segregation and is crucial for the formation of discrete, individualized bivalent chromosomes.

Introduction

Meiotic chromosomes must undergo a series of structural and organizational changes to achieve the partitioning of homologues and then sister chromatids required for the production of haploid gametes. Faithful segregation requires that chromosomes be compacted, resolved from one another, and reorganized to permit bipolar spindle attachment. This paper establishes the role of the *Caenorhabditis elegans* condensin complex in the molecular machinery that underlies the condensation and restructuring of meiotic chromosomes.

The restructuring of chromosomes during prophase I facilitates two key events of meiosis: the reciprocal exchange of DNA between homologues (crossover recombination), and chromosome segregation (for review see Moore and Orr-Weaver, 1998; Zickler and Kleckner, 1999). The pairing and lengthwise alignment (synapsis) of each set of homologues in leptotene/zygotene are crucial for recombination in pachytene. After pachytene exit, homologues begin to separate (desynapsis); the desynapsing chromosomes commonly undergo a transient period of decondensation. These homologues are again reorganized in diplotene and diakinesis in preparation for their segregation in anaphase I. During this reorganization in *C. elegans*, chromosomes undergo rapid condensation that often accounts

for most of the DNA compaction in meiosis. Additionally, sister chromatids are restructured around the site of crossover to form a compact, cross-shaped structure, hereafter referred to as a cruciform bivalent or a diakinesis bivalent. DNA condensation and chromatid restructuring are both critical for successful chromosome segregation.

From a functional standpoint, DNA compaction in late prophase may fulfill several important roles for chromosome segregation. Chromosome condensation aids the unencumbered movement of chromosomes in metaphase and anaphase and reduces the likelihood of chromosome entrapment during cytokinesis (Swedlow and Hirano, 2003). Compaction also helps drive the resolution of chromosomes required for segregation (Holm, 1994; Koshland and Strunnikov, 1996; Swedlow and Hirano, 2003). For example, the disentanglement of two intertwined DNA duplexes by enzymes such as topoisomerase II requires a second activity (compaction or poleward forces) to hold the newly disentangled DNA duplexes apart (Holm, 1994). Otherwise, these enzymes could just as easily reintroduce catenates between adjacent DNA duplexes. Here, we identify DNA restructuring factors required for both DNA compaction and sister chromatid resolution, and show that DNA compaction alone is not sufficient to achieve sister chromatid resolution.

Chromosome-restructuring factors that function specifically in the remodeling of chromosomes after pachytene exit have been difficult to recognize because mutations that affect steps in early prophase are likely to disrupt the formation of cruciform bivalents later in prophase without affecting the

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The online version of this article includes supplemental material.

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Abbreviations used in this paper: CAP, chromosome-associated polypeptide; DSB, double-strand break; HCP, holocentric chromosome-binding protein; IP, immunoprecipitation; NEBD, nuclear envelope breakdown; RNAi, RNA interference; SC, synaptonemal complex; SMC, structural maintenance of chromosomes.

process directly. Nonetheless, candidates exist for such chromosome-restructuring factors and include members of the conserved condensin protein complexes. In mitosis, condensin activity is required to compact and resolve chromosomes for accurate chromosome segregation (for review see Nasmyth, 2002; Swedlow and Hirano, 2003). Despite the need for structural changes in meiotic as well as mitotic chromosomes, a direct role for condensin in the transition of a homologue pair into an ordered diakinesis bivalent has not been demonstrated. A role for condensin earlier in meiotic prophase has been shown for *Saccharomyces cerevisiae*, in which condensin mutations affected pachytene chromosome morphology and synapsis (Yu and Koshland, 2003). Because these early defects also disrupted late prophase chromosome morphology, a subsequent role for condensin could not be defined in *S. cerevisiae*. Given the meiotic chromosome compaction that accompanies the formation of cruciform bivalents, we analyzed the DNA localization and functional requirements of *C. elegans* condensin in meiotic prophase I.

The prototypical condensin complex contains at least five subunits, including a pair of structural maintenance of chromosomes (SMC) proteins (SMC2 and SMC4) and three non-SMC proteins that belong to the chromosome-associated polypeptide (CAP) CAP-D2, CAP-G, and CAP-H/Barren families (Swedlow and Hirano, 2003). Two separate mitotic condensin complexes have been identified in many organisms; they share SMC components but have unique non-SMC components (Ono et al., 2003; Yeong et al., 2003). At least two condensin-like complexes exist in *C. elegans*; however, one functions in the sex-specific process of X chromosome dosage compensation (Chuang et al., 1994; Lieb et al., 1996, 1998), and the other functions in chromosome segregation during mitosis and meiosis (Lieb et al., 1998; Hagstrom et al., 2002). MIX-1, the single *C. elegans* SMC2 homologue, is essential for both processes and can be found in both complexes (Lieb et al., 1998; Hagstrom et al., 2002). In contrast, each of the two SMC4 homologues, DPY-27 and SMC-4, functions exclusively in a single process: DPY-27 in dosage compensation and SMC-4 in chromosome segregation (Chuang et al., 1994; Hagstrom et al., 2002; Kaitna et al., 2002). DPY-26, a CAP-H/Barren homologue, participates in the dosage compensation complex (Lieb et al., 1996).

To define additional condensin proteins and to explore the role of the complex in meiosis, we biochemically defined factors associated with MIX-1. We identified holocentric chromosome-binding protein 6 (HCP-6; Stear and Roth, 2002) as a non-SMC component of the MIX-1/SMC-4 condensin complex, and we showed HCP-6 to be essential for both meiotic divisions. Surprisingly, we found condensin to have different requirements for its assembly onto mitotic versus meiotic chromosomes. Condensin was first detected on meiotic DNA after pachytene exit, when it colocalized with sister chromatids. Consistent with its localization, condensin functions in diplotene and diakinesis as a chromosome-restructuring complex that organizes pairs of desynapsing homologues into compact, well-resolved cruciform bivalents. Finally, condensin helps to resolve or prevent cohesin-independent linkages between sister chromatids and between homologues before metaphase I, allowing accurate chromosome segregation.

Results

HCP-6 is a homologue of CAP-D3 and a component of the *C. elegans* condensin II complex

MIX-1, the *C. elegans* SMC2 homologue, mediates both dosage compensation and mitotic chromosome condensation through its participation in two different condensin-like complexes (Lieb et al., 1998; Hagstrom et al., 2002). To identify non-SMC partners for MIX-1 in either complex, we immunoprecipitated both complexes from embryonic extracts using MIX-1 antibodies (Fig. 1 A). Microsequencing of proteolytic peptides from individual protein bands in the MIX-1 immunoprecipitation (IP) identified the expected dosage compensation protein DPY-27, the expected mitotic condensin subunit SMC-4, and two additional proteins (predicted products from ORFs Y39A1B.3 and Y110A7A.1). Y39A1B.3 (M_r of 160 kD) encodes the dosage compensation protein DPY-28, a homologue of the condensin I non-SMC subunit CAP-D2 (Fig. 1 D; Plenefisch et al., 1989; Tsai, C., M. Albrecht, and B. Meyer, personal communication). Y110A7A.1 (M_r of 200 kD) encodes HCP-6, a homologue of the condensin II non-SMC subunit CAP-D3 (Fig. 1 D; Ono et al., 2003; Yeong et al., 2003). HCP-6 is required for mitotic chromosome segregation (Stear and Roth, 2002). Western blot analysis confirmed the presence of all four microsequenced proteins in MIX-1 IPs and also identified the expected dosage compensation protein DPY-26 (Fig. 1 B, lane 1). The interaction of MIX-1 with HCP-6 and DPY-28 was confirmed by reciprocal IP reactions in which DPY-28 and HCP-6 antibodies precipitated MIX-1 (Fig. 1 B, lanes 2 and 3).

DPY-28 and HCP-6 function exclusively in two separate complexes: the dosage compensation complex subunits DPY-26 and DPY-27 were detected only in the DPY-28 IP, whereas the condensin subunit SMC-4 was found only in the HCP-6 IP (Fig. 1 B, lanes 2 and 3). DPY-28 and HCP-6 are not functionally redundant during embryogenesis because both proteins are essential for viability and the terminal mutant phenotypes are distinct (see below; Plenefisch et al., 1989; Stear and Roth, 2002). The similarities of DPY-28 to CAP-D2 and DPY-26 to the condensin I subunit CAP-H/Barren (Lieb et al., 1996) suggest that a condensin-I-like complex was preserved in *C. elegans* and coopted to implement dosage compensation. The similarity of HCP-6 to condensin II subunit CAP-D3 and the data below indicate that HCP-6 mediates chromosome resolution, condensation, and segregation during mitotic and meiotic divisions through its participation in a condensin II-like complex.

HCP-6 and MIX-1 colocalize on centromeres of mitotic chromosomes and are required for mitotic chromosome segregation

HCP-6 and MIX-1 colocalize with the centromeric histone variant CENP-A on the poleward faces of metaphase chromosomes during mitotic divisions in embryos and in the germline (Fig. 1 E and Fig. 2 A; Hagstrom et al., 2002; Stear and Roth, 2002). This pattern of localization and the biochemistry above indicate that HCP-6 and MIX-1 form a complex that associates with cen-

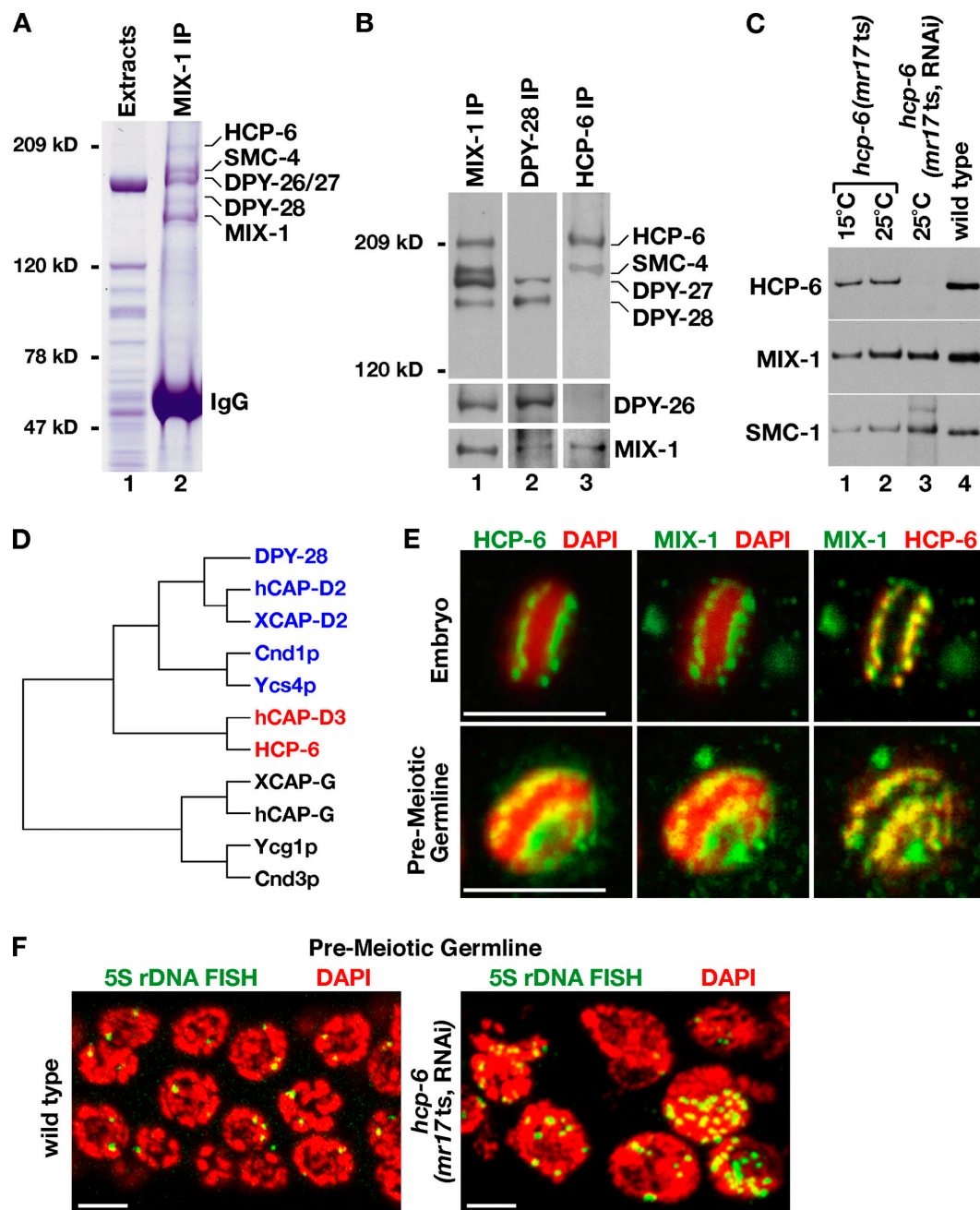
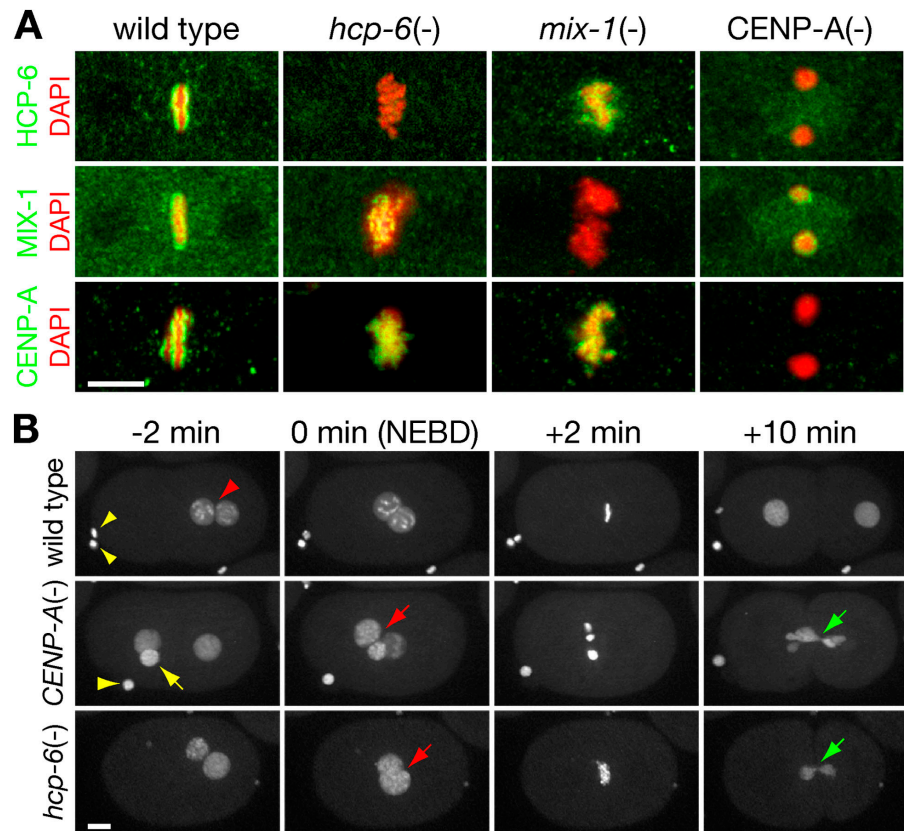


Figure 1. HCP-6 associates exclusively with the mitotic condensin II complex and colocalizes with MIX-1 on mitotic chromosomes. (A) Coomassie staining and microsequencing identified proteins in MIX-1 IPs. (B) Western blot analysis of MIX-1, DPY-28, and HCP-6 IPs confirmed association of DPY-28 and HCP-6 with MIX-1. Dosage compensation proteins DPY-26 and DPY-27 were found only in DPY-28 and MIX-1 IPs (lane 2), and mitotic condensin subunit SMC-4 only in HCP-6 and MIX-1 IPs (lane 3). Blots were probed with mixtures of antibodies. (C) HCP-6 protein levels were not reduced in *hcp-6(mr17)* mutants relative to the MIX-1 and SMC-1 loading controls, but HCP-6 was undetectable in *hcp-6(mr17)* RNAi mutants. (D) Phylogenetic tree comparing DPY-28, HCP-6, and CAP-D2, CAP-D3, and CAP-G homologues. HCP-6 is closest to CAP-D3 of condensin II. (E) HCP-6 and MIX-1 colocalized on metaphase chromosomes in embryos and the premeiotic germline. (F) 5S rDNA FISH revealed aneuploid nuclei in the premeiotic germline of *hcp-6* mutant hermaphrodites. Bars, 5 μ m.

romeres of mitotic chromosomes. To further define the roles for condensin in mitosis, we identified conditions that severely reduce HCP-6 function. The *hcp-6(mr17)* allele has a missense mutation (Stear and Roth, 2002) that results in temperature-sensitive embryonic lethality (Table S1, available at <http://www.jcb.org/cgi/content/full/jcb.200408061/DC1>); however, HCP-6 protein levels are not reduced (Fig. 1 C, lanes 1 and 2).

Therefore, we treated *hcp-6(mr17)* mutants with *hcp-6* RNA interference (RNAi) to deplete HCP-6 to levels undetectable by Western blot analysis (Fig. 1 C, lane 3) and immunostaining (Fig. 2 A, Fig. 5 E). Any residual protein would be compromised by the *hcp-6(mr17)* mutation. MIX-1 function was similarly reduced by *mix-1* RNAi in worms homozygous for the maternal-effect embryonic lethal allele *mix-1(b285)* (Fig. 2 A, Fig. 5 E).

Figure 2. HCP-6 and MIX-1 associate independently with mitotic chromosomes and are required for chromosome condensation and segregation. (A) HCP-6 was undetectable in *hcp-6(mr17, RNAi)* mutant embryos, yet MIX-1 and CENP-A still associated with chromosomes at the disorganized metaphase plate. Similarly, MIX-1 was undetectable in *mix-1(b285, RNAi)* embryos, but HCP-6 and CENP-A still accumulated on chromosomes. HCP-6 did not associate with chromosomes of embryos depleted for two CENP-A paralogues, but MIX-1 did. (B) Mitotic chromosomes tagged with GFP::H2B histone condensed in pronuclei of wild-type embryos (red arrowhead) at least 2 min before NEBD, but decondensed chromosomes persisted until NEBD in pronuclei of *hcp-6(mr17)* and CENP-A-depleted embryos (red arrows). Defective mitotic chromosome segregation resulted in the formation of anaphase bridges (green arrows). Two polar bodies were extruded during meiosis in wild-type embryos (yellow arrowheads), but extra pronuclei formed in CENP-A-depleted embryos (yellow arrow) due to meiotic defects. Bars, 5 μ m.



Reducing HCP-6 function disrupted mitotic chromosome segregation in embryos (Fig. 2 B; Stear and Roth, 2002) and in the germline (Fig. 1 F). Nuclei in the wild-type premeiotic germline are typically uniform in size, but equivalent regions of *hcp-6(mr17)* and *hcp-6(mr17, RNAi)* mutants contained predominantly macro- and micro-nuclei. 5S rDNA FISH showed extensive aneuploidy in these nuclei (Fig. 1 F), a hallmark of defective chromosome segregation.

SMC and non-SMC condensin subunits can associate with chromatin independently

Recent models propose that the condensin complex associates with chromosomes as a ring that encircles DNA (Yoshimura et al., 2002; Hopfner and Tainer, 2003). These models suggest that loss of any single condensin subunit might disrupt the association of other condensin components with DNA. Indeed, the intact condensin complex can associate with chromatin in *Xenopus* egg extracts and in budding yeast cells, whereas subcomplexes containing only the SMC or non-SMC subunits cannot (Kimura and Hirano, 2000; Lavoie et al., 2002). To determine whether an intact condensin complex is required for association of individual condensin subunits with *C. elegans* chromosomes, we examined the loading dependencies of HCP-6 and MIX-1. Depletion of either condensin subunit resulted in similar chromosome segregation defects, but the other subunit still loaded (Fig. 2 A). HCP-6 associated with the disorganized chromosomes of *mix-1(b285, RNAi)* embryos, and MIX-1 accumulated on chromosomes of *hcp-6(mr17, RNAi)* embryos (Fig. 2

A). Similarly, MIX-1 accumulated on chromosomes of CENP-A-depleted embryos (Fig. 2 A), even though the association of HCP-6 was severely disrupted (Fig. 2 A; Stear and Roth, 2002). Thus, both SMC and non-SMC condensin subunits are retained on chromosomes without their stoichiometric interaction with other condensin subunits, suggesting that a closed ring structure may not be essential for the stable association of individual condensin components with mitotic chromosomes.

The requirement for CENP-A in HCP-6 loading suggests a role for CENP-A in chromosome condensation. Such a requirement has not been described in *C. elegans*, despite the extensive characterization of CENP-A's role in chromosome segregation (Buchwitz et al., 1999; Oegema et al., 2001). We show that although chromosome condensation occurs in wild-type embryos at least 2 min before nuclear envelope breakdown (NEBD), diffuse chromosomes persist in both CENP-A-depleted embryos and *hcp-6* mutants until NEBD (Fig. 2 B). Thus, CENP-A, like HCP-6, facilitates chromosome condensation in mitotic prophase.

HCP-6 is required for chromosome segregation in meiosis I and II

Chromosome condensation is essential for chromosome segregation in mitosis and is a conserved feature of chromosome segregation in meiosis, yet a conserved role for condensin in meiosis has not been established. Therefore, we assessed the requirement for condensin in meiosis I and II of *C. elegans* in animals with chromosomes tagged by a GFP::H2B histone (Praitis et al., 2001). Prominent DNA bridges formed between

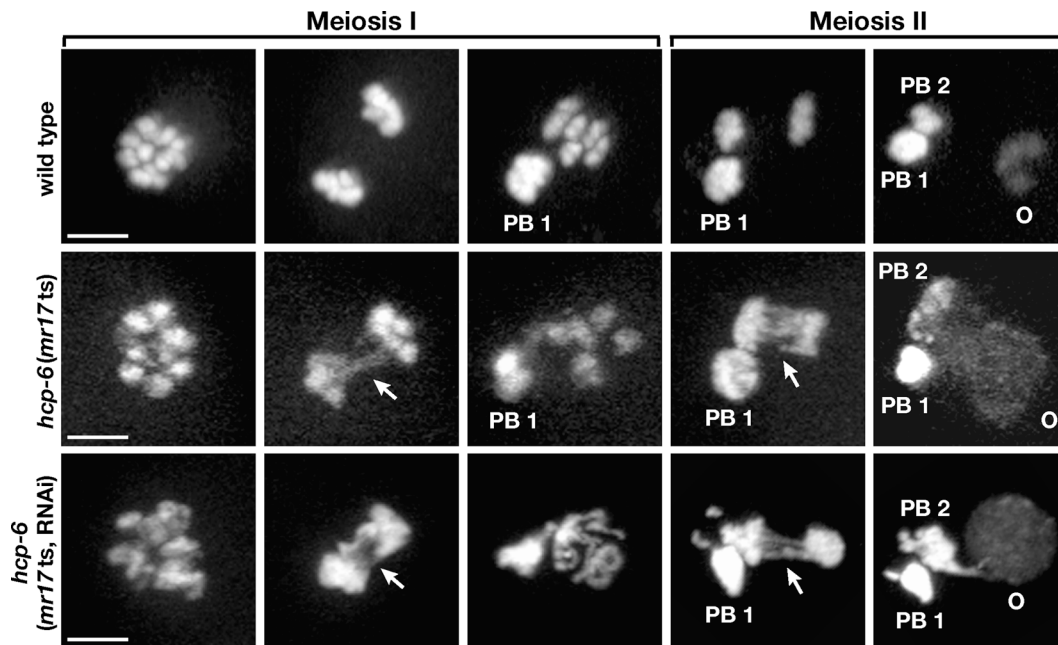


Figure 3. **HCP-6 is required for chromosome segregation in meiosis I and II.** In wild-type zygotes, GFP::H2B histone-tagged chromosomes align on the metaphase plate after breakdown of the oocyte nuclear envelope. Homologues separate in anaphase of meiosis I; one set is extruded into the first polar body (PB1). Sister chromatids separate in meiosis II; one set is extruded into the second polar body (PB2). The second set decondenses and forms the oocyte pronucleus (O). In *hcp-6(mr17)* and *hcp-6(mr17, RNAi)* mutants, DNA bridges connected separating chromosomes in anaphase I and II (arrows). Bars, 5 μ m.

segregating chromosomes during anaphase I and anaphase II in all *hcp-6(mr17, RNAi)* zygotes observed ($n = 15$; Fig. 3). Chromatin bridges also formed in all *hcp-6(mr17)* zygotes, but were consistently less severe in anaphase I than in anaphase II ($n = 9$; Fig. 3). Chromosome segregation defects were detected during meiosis II (but not meiosis I) in animals treated with RNAi to deplete HCP-6 (unpublished data), consistent with the report that only anaphase II was affected by RNAi depletion of MIX-1 or SMC-4 (Hagstrom et al., 2002). Thus, condensin is essential for homologue segregation in meiosis I, but this segregation appears less sensitive to condensin depletion than sister chromatid segregation in meiosis II. The combined results from worms, yeast (Yu and Koshland, 2003), and plants (Siddiqui et al., 2003) indicate a conserved requirement for condensin activity in both meiotic divisions.

Description of meiotic prophase in the wild-type germline

To discern the basis for the meiotic DNA bridges caused by reduced condensin function, we examined condensin localization in wild-type animals and performed a detailed analysis of meiotic prophase I in condensin mutants. Context for this analysis is set by the following description.

In the *C. elegans* germline, meiotic nuclei are arranged in a temporal-spatial order, revealing the dramatic morphological changes that occur during meiosis I prophase. The premeiotic germline, which lies at the distal end of the gonad, contains mitotically proliferating nuclei and nuclei in premeiotic S-phase. Pairing and alignment of homologues initiate downstream in the gonad, in the "transition zone," the equivalent of leptotene-zygotene. In pachytene, homologues are fully synapsed (Fig. 4),

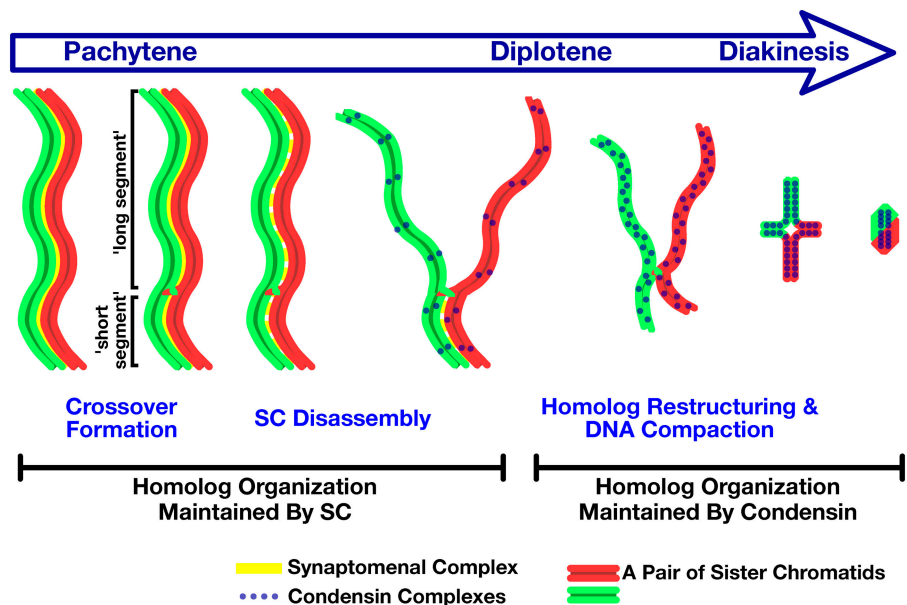
are distinct from one another, and appear in DAPI-stained nuclei as discrete, parallel tracks separated by a narrow gap occupied by the synaptonemal complex (SC). A single crossover forms during pachytene between each pair of homologues. The SC disassembles as nuclei exit pachytene and enter diplotene, but desynapsed homologues remain tethered by sister chromatid cohesion flanking the crossover (Fig. 4). Cellularized oocytes pause in diakinesis of prophase I with condensed homologue pairs until fertilization, the event that triggers meiosis I and II (McCarter et al., 1999).

The events of diplotene-diakinesis prepare chromosomes for their segregation later in meiosis: chromosomes compact and are reorganized around the crossover site to form highly condensed, cruciform bivalents (Fig. 4; Albertson et al., 1997). The crossover site, which occurs typically in the terminal one-third of each chromosome, dividing the chromosome asymmetrically into short and long segments, specifies the long and short arms of the diakinesis bivalents (Albertson et al., 1997). The short arms form the interface where the two recombined homologues are linked by the cohesin complex (Fig. 4; Albertson et al., 1997; Pasierbek et al., 2001; Chan et al., 2003). Diakinesis bivalents must be condensed, resolved, and reorganized into a structure that facilitates the formation of bipolar spindle attachments.

Male meiosis resembles meiosis in hermaphrodites, except a state equivalent to diplotene-diakinesis is not apparent. Instead, chromosomes condense rapidly after pachytene, proceed through meiosis I and II, and are packaged into sperm.

We show that condensin is essential in late meiotic prophase for chromosome compaction and for the formation of discrete, resolved diakinesis bivalents. Because the meiotic

Figure 4. **Homologues undergo dramatic structural reorganization during prophase of meiosis I.** This diagram depicts the progression of a pair of homologues through prophase I. Homologue pairing and alignment initiate in leptotene–zygotene (not depicted). In pachytene, SC (yellow line) stabilizes homologue synapsis (red and green lines). A single crossover typically divides each homologue pair into long and short segments. SC disassembly in diplotene results first in desynapsis of the long segment of each homologue pair and then of the short segment (Nabeshima, K., M. Colaiácovo, and A. Villeneuve, personal communication). Recombinant homologues are reorganized around the crossover site and condensed to form highly compacted, cruciform bivalents. The short segment becomes the short arm of the diakinesis bivalent, where cohesin-mediated linkages maintain the association of homologues. Importantly, HCP-6 and MIX-1 (blue dots) become enriched on chromosomes after pachytene exit, and our evidence indicates that both proteins are required for the reorganization of homologues and formation of discrete, compacted diakinesis bivalents.



prophase defects in condensin mutants occurred before spindle assembly, they were not the result of merotelic attachments, in which a single kinetochore attaches to microtubules from both poles, or syntelic attachments, in which the kinetochores of both homologues attach to a single pole.

HCP-6 and MIX-1 localize to sister chromatids during diplotene–diakinesis of meiotic prophase I

The localization of HCP-6 and MIX-1 was examined in wild-type gonads to assess when condensin first associates with meiotic chromosomes to initiate the structural changes essential for chromosome segregation. In both sexes, HCP-6 was detected in nuclei throughout meiosis, but HCP-6 was excluded from the chromosomes of transition zone and pachytene nuclei (Fig. 5, A and B). In males, HCP-6 was first detected on chromosomes as they compacted after pachytene and congressed to the metaphase plate, and HCP-6 staining persisted on DNA through meiosis II (Fig. S1 A, available at <http://www.jcb.org/cgi/content/full/jcb.200408061/DC1>). In hermaphrodites, HCP-6 first accumulated on DNA in diplotene (Fig. S1 B). By diakinesis, each bivalent contained four discrete HCP-6 foci (Fig. 5 C), which marked the four sister chromatids as demonstrated by two experiments. First, the interface between the four HCP-6 foci was delineated by cohesin subunit SMC-1 (Fig. 5 C), which localizes in a cruciform pattern that marks the boundaries between sister chromatids (Pasierbek et al., 2001; Chan et al., 2003). Second, many homologues persist in diakinesis as two separate univalents in animals partially depleted of the meiosis-specific cohesin subunit REC-8 (Fig. 5 D; Pasierbek et al., 2001; Chan et al., 2003). Each univalent contains two sister chromatids, and two distinct HCP-6 foci were present on 65% of DAPI-staining bodies in diakinesis nuclei of *rec-8(RNAi)* animals ($n = 49$).

Condensin subunit MIX-1 was also detected on chromosomes in diplotene and was present in four foci on diakinesis bivalents (Fig. 5 C; Fig. S1 B). We conclude that the condensin complex is first enriched on meiotic chromosomes in diplotene and localizes to individual sister chromatids at diakinesis.

Different requirements for condensin binding to mitotic and meiotic chromosomes

Surprisingly, we found different requirements for the chromosomal association of condensin subunits in meiosis and mitosis (Fig. 2 A, Fig. 5 E). Although HCP-6 and MIX-1 associate independently with mitotic chromosomes (Fig. 2 A), MIX-1 depends on HCP-6 for its association with meiotic chromosomes (Fig. 5 E). Additionally, although HCP-6 localization and chromosome condensation in mitosis required CENP-A (Fig. 2 A), both HCP-6 and MIX-1 associated with meiotic chromosomes in CENP-A–depleted animals, and chromosomes appeared condensed (Fig. 5 E). We conclude that different rules govern the loading or retention of condensin subunits on chromosomes during meiotic and mitotic divisions.

Condensin is required in diplotene–diakinesis for chromosome compaction and formation of discrete diakinesis bivalents

The timing of HCP-6 and MIX-1 accumulation on chromosomes suggested that meiotic condensin is first required in diplotene–diakinesis, when chromosomes compact to form highly condensed, cruciform bivalents (Albertson et al., 1997). To determine whether condensin acts at this time, we examined the requirements for condensin in meiotic prophase using conditions that inactivated meiotic condensin without causing extensive aneuploidy in meiotic regions of the germline (see Materials and methods).

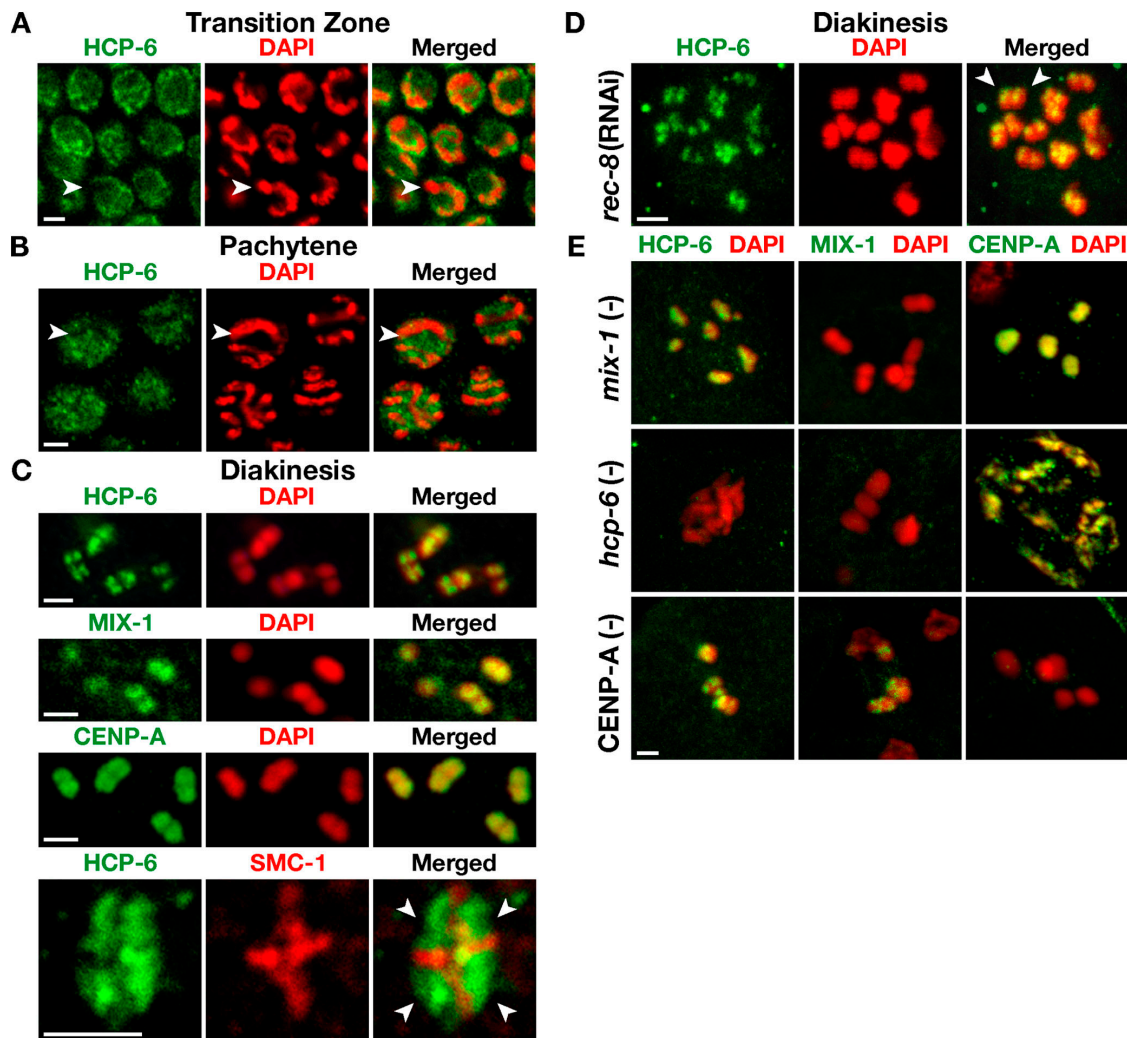


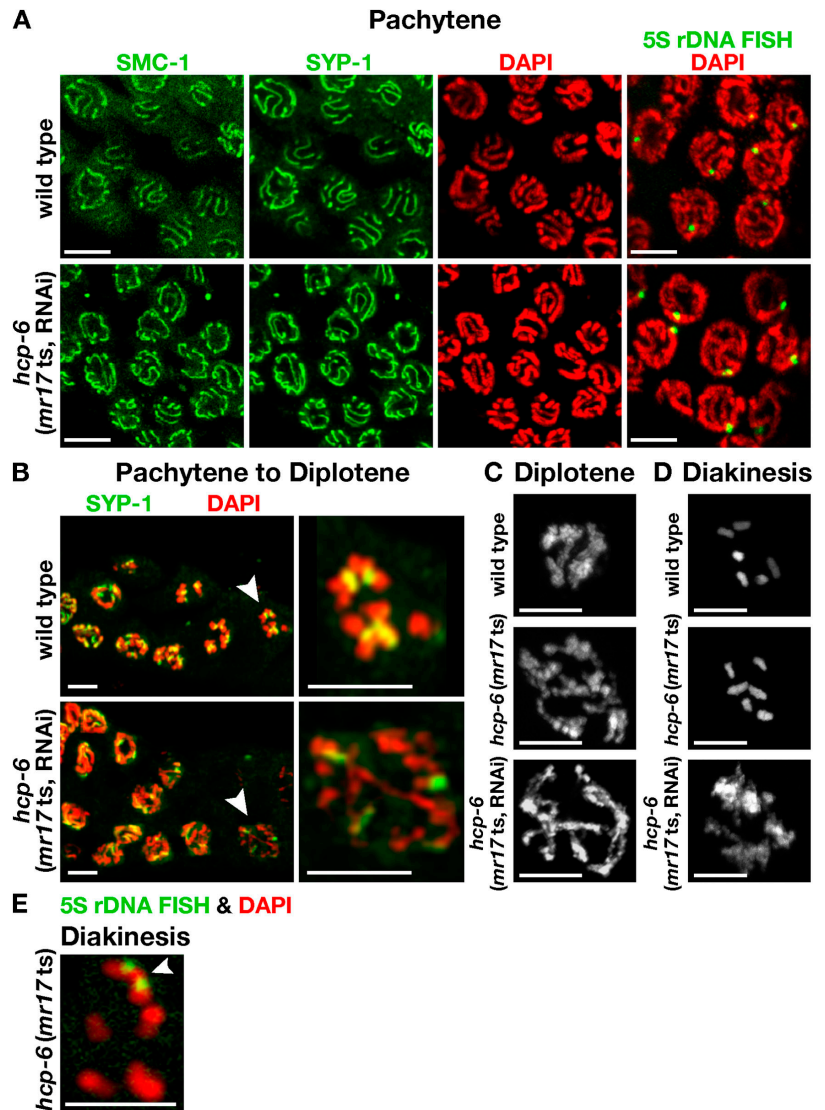
Figure 5. HCP-6 and MIX-1 first associate with meiotic chromosomes at diplotene–diakinesis. In confocal images of wild-type transition zone (A) and pachytene (B) nuclei, HCP-6 appeared nucleoplasmic but excluded from DNA (arrowheads indicate regions devoid of HCP-6 staining). (C) HCP-6 and MIX-1 accumulate in four quadrants on wild-type diakinesis bivalents. CENP-A was also present in four foci per bivalent, but these foci were broader than the HCP-6 and MIX-1 foci. The four HCP-6 foci (fourth row, arrowheads) were bisected by SMC-1 staining, indicating that each quadrant represents one sister chromatid. (D) After partial depletion of REC-8, two HCP-6 foci (arrowheads) were present on each univalent, correlating with the presence of two sister chromatids per univalent. (E) MIX-1 was undetectable on bivalents in *mix-1(b285, RNAi)* mutants, but HCP-6 still accumulated on chromosomes. In contrast, MIX-1 required HCP-6 for its association with meiotic chromosomes. MIX-1 was undetectable on bivalents in animal mutants for the partial loss-of-function allele *hcp-6(mr17)*, which also disrupted HCP-6 loading. Association of MIX-1 with the dosage compensation complex does not require HCP-6: MIX-1 antibodies stained X chromosomes in gut nuclei of *hcp-6(mr17)* and *hcp-6(mr17, RNAi)* mutants (not depicted). Both MIX-1 and HCP-6 loaded on meiotic chromosomes in CENP-A depleted worms, and CENP-A localized on chromosomes in *mix-1(b285, RNAi)* and *hcp-6(mr17, RNAi)* oocytes. Bars, 2 μ m.

Pachytene chromosome organization appeared normal in *hcp-6* mutants (Fig. 6 A), consistent with the association of HCP-6 with chromosomes after pachytene exit. 5S rDNA FISH indicated that synapsis was unaffected by *hcp-6* inactivation. Moreover, SC central element SYP-1 and cohesin subunits SMC-1 and SMC-3 localized between homologues, as in wild type (Fig. 6 A, Fig. 7 A). In *hcp-6(mr17)* and *hcp-6(mr17, RNAi)* animals, homologue pairs appeared normally compacted and had no obvious attachments to other homologue pairs in late pachytene nuclei (Fig. 6 A; unpublished data). Finally, linkages between homologues were resolved because *rec-8(RNAi)* caused similar levels of asynapsis in late pachytene nuclei of *hcp-6(mr17)* animals grown at 15 and 25°C (Fig. 8 A). Both the timing of condensin localization

and the phenotypes of condensin mutants suggest that factors other than HCP-6 mediate the condensation, individualization, and resolution of chromosomes accompanying entry into pachytene. However, we cannot exclude the possibility that residual condensin function is sufficient for these processes.

Some features of pachytene exit also appeared normal in *hcp-6(mr17)* and *hcp-6(mr17, RNAi)* mutants. SYP-1 staining diminished along each pair of desynapsing homologues in diplotene, indicating that the timing of SC disassembly was unaffected by *hcp-6* disruption (Fig. 6 B). Additionally, a short stretch of SYP-1 staining persisted on each homologue pair until late diakinesis, as in wild type (Fig. 6 B, arrowhead; MacQueen et al., 2002).

Figure 6. HCP-6 mediates chromosome compaction and resolution at diplotene–diakinesis. (A) Confocal images of DAPI staining and 5S rDNA FISH revealed normal DNA morphology and homologue association in pachytene of *hcp-6* mutants. Moreover, staining of cohesin subunit SMC-1 and SC central element SYP-1 were indistinguishable in wild-type and *hcp-6* mutant pachytene nuclei. (B) Residual SYP-1 staining in late pachytene and diplotene nuclei was similar in wild-type and *hcp-6(mr17, RNAi)* animals (left column, arrowhead), suggesting normal SC disassembly in *hcp-6* mutants. However, wild-type chromosomes compacted rapidly in diplotene but *hcp-6* mutant chromosomes were decondensed (right column, magnified images of nuclei marked with arrowheads in left column). (C) Diplotene chromosomes decondensed in *hcp-6(mr17, RNAi)* and *hcp-6(mr17)* mutants. (D) Resolution of diakinesis bivalents failed in *hcp-6(mr17, RNAi)* animals. Six compact bivalents were resolved by late diakinesis in *hcp-6(mr17)* mutants, as in wild type (see Fig. 8 E). (E) 5S rDNA FISH indicated that homologue realignment of chromosome V (arrowhead) was achieved by diakinesis in *hcp-6(mr17)* mutants. Bars, 5 μ m.



In contrast, obvious defects in chromosome compaction and organization occurred during diplotene and diakinesis in *hcp-6(mr17, RNAi)* mutants. Diplotene chromosomes decondensed during SC disassembly and often appeared elongated and threadlike (Fig. 6, B and C), in contrast to chromosomes of wild-type animals, which condensed rapidly during SC disassembly (Fig. 6, B and C). This decompaction was even more pronounced in diakinesis (Fig. 6 D). Although six discrete, highly compacted bivalents were present in wild-type diakinesis nuclei, similarly staged nuclei of *hcp-6(mr17, RNAi)* mutants had decondensed chromosomes not separated from one another (Fig. 6 D). Thus, HCP-6 is essential for the overall compaction of chromosomes in diplotene and diakinesis and for the formation of well-resolved diakinesis bivalents.

The phenotype of *hcp-6(mr17)* mutants was less severe than that of *hcp-6(mr17, RNAi)* animals. Chromosomes transiently decondensed after SC disassembly (Fig. 6 C), and formation of compacted, discrete bivalents was delayed. Compact individualized bivalents appeared late in diakinesis of *hcp-6(mr17)* mutants, rather than early, as in wild-type animals

(Fig. 6 D, Fig. 8 E). A delay was also found in *mix-1(b285)* mutants (unpublished data), further establishing that the condensin complex, and not just HCP-6, is required for both chromosome compaction and the formation of resolved bivalents in diplotene–diakinesis.

Despite the delay in the formation of discrete bivalents in *hcp-6(mr17)* mutants, late diakinesis bivalents resembled those of wild type. Realignment of *hcp-6(mr17)* homologues was achieved: FISH analysis showed two distinct 5S rDNA loci, demonstrating the separation of the long arms of chromosome V in *hcp-6* mutants as in wild type (Fig. 6 E). Moreover, the Aurora B kinase homologue AIR-2 localized to the mid-bivalent in the oldest oocyte of *hcp-6(mr17)* gonads (81% of oocytes, $n = 70$), as in wild-type gonads (78% of oocytes, $n = 58$; Fig. 7 A; Fig. S1 C). AIR-2 facilitates the cleavage of REC-8 along the short arms, which permits homologue separation in anaphase I (Rogers et al., 2002). Consistent with the proper localization and function of AIR-2, one of its substrates, serine 10 of histone H3, was phosphorylated in the mid-bivalent of *hcp-6(mr17)* mutants (Fig. S1 D). Finally, oocyte cellulariza-

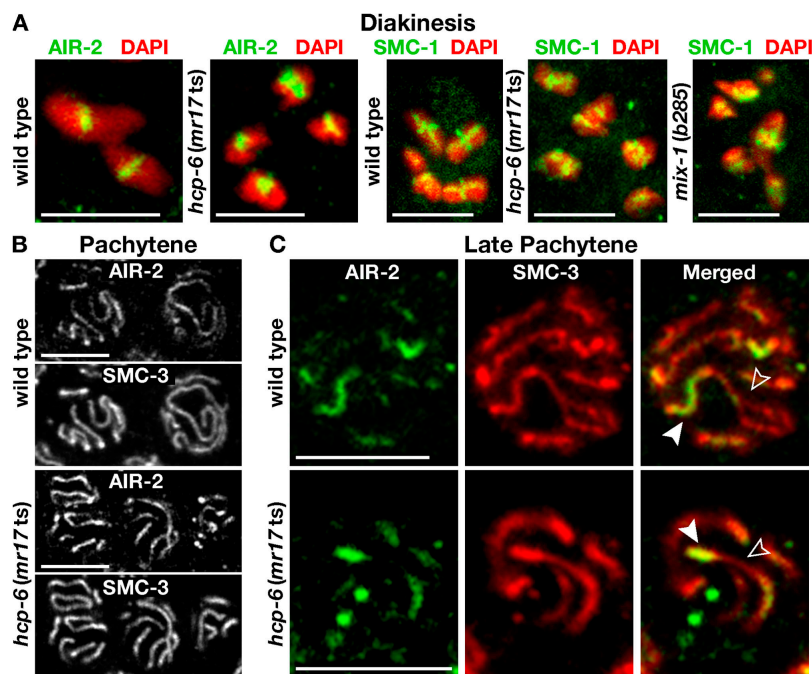


Figure 7. Condensin is required for chromosome organization within diakinesis bivalents. (A) AIR-2 localized at the midbivalent in wild-type and *hcp-6(mr17)* animals. In contrast, SMC-1 adopted a cruciform pattern in wild-type bivalents, but was disorganized in *hcp-6(mr17)* and *mix-1(b285)* bivalents. Each panel shows a representative subset of bivalents in a single diakinesis nucleus. (B) AIR-2 and SMC-3 colocalized along the length of synapsed pachytene homologues in wild-type and *hcp-6(mr17)* animals. (C) During SC disassembly, AIR-2 diminished along the desynapsed region of each homologue pair. AIR-2 persisted along the synapsed region (solid arrowhead). SMC-3 staining persisted along the entire length of homologue pairs, revealing the separation of desynapsed regions (hollow arrowhead). Bars, 5 μm.

tion occurred relatively normally in *hcp-6(mr17)* mutants (Table S2, available at <http://www.jcb.org/cgi/content/full/jcb.200408061/DC1>). Because *hcp-6(mr17)* does not disrupt the ultimate compaction and reorientation of chromosomes into discrete diakinesis bivalents, we could ask whether compaction alone imparts proper structural organization to individual sister chromatids within a diakinesis bivalent.

DNA compaction is not sufficient to properly order diakinesis bivalents

Although aspects of bivalent organization appeared normal in diakinesis nuclei of *hcp-6(mr17)* mutants, as indicated by AIR-2 localization, histone H3 phosphorylation, and the realignment of homologues, the organization of sister chromatids within each half-bivalent was aberrant. A cruciform pattern of cohesin localization was detected in only 5–6% of *hcp-6(mr17)* oocytes stained with SMC-1 ($n = 121$) or SMC-3 ($n = 254$) antibodies, compared with 74–76% of wild-type diakinesis oocytes stained with SMC-1 ($n = 122$) or SMC-3 ($n = 194$) antibodies (Fig. 5 C, Fig. 7 A, and Fig. S1, C and D). Cohesin appeared disorganized and dispersed throughout the chromosomes of *hcp-6(mr17)* and *mix-1(b285)* diakinesis bivalents (Fig. 7 A), including the mid-bivalent region (Fig. S1 C) where AIR-2 staining looks fairly normal. The aberrant staining implies that proper organization of sister chromatids had not been achieved in the mutant bivalents. Because the bivalents in *hcp-6(mr17)* and *mix-1(b285)* mutants condensed to the same size and shape as bivalents in wild-type animals, condensation alone does not guarantee proper sister chromatid structure.

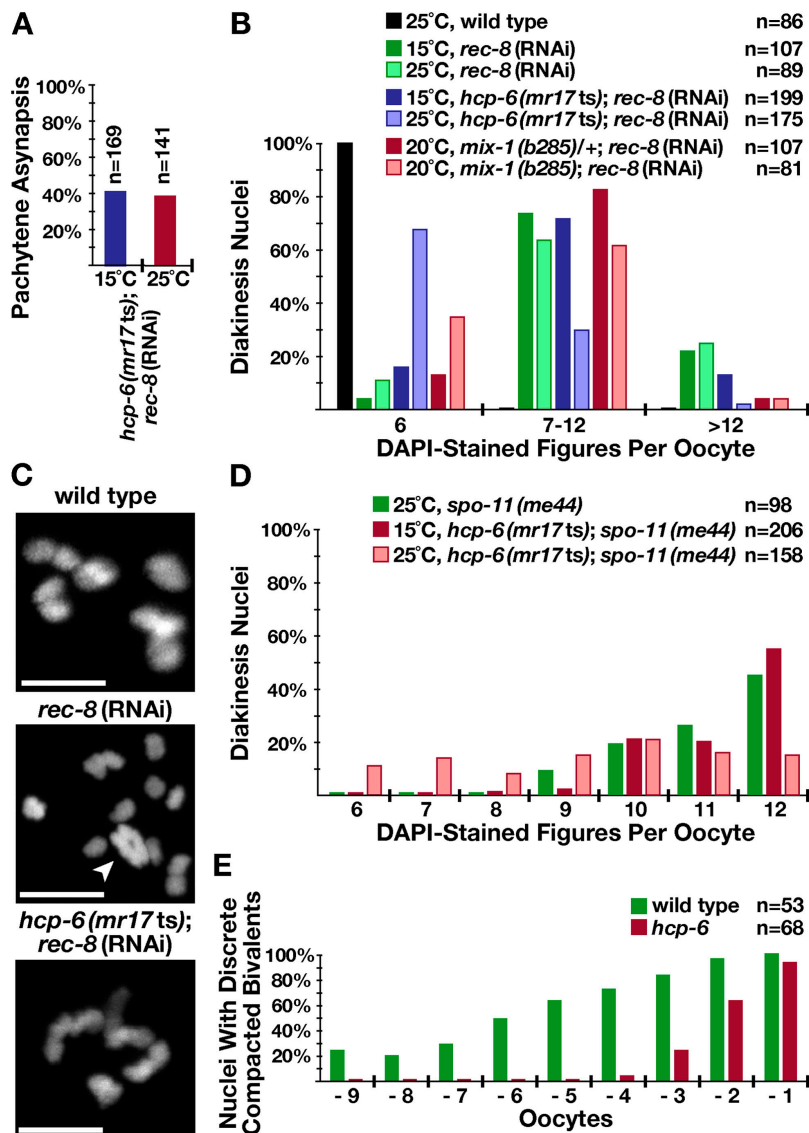
In light of the abnormal cohesin pattern in late diakinesis nuclei of *hcp-6* mutants, it was surprising that AIR-2 pattern was reasonably normal in the oldest oocyte. To investigate how this difference arose, we compared the localization of AIR-2 and SMC-3 throughout meiotic prophase (Fig. 7, B and C;

Fig. S1 E). We detected AIR-2 earlier in prophase than previously reported (Rogers et al., 2002). In pachytene nuclei, AIR-2 colocalized with SMC-3 and the SC along the length of synapsed homologues (Fig. 7 B; Nabeshima, K., M. Colaiácovo, and A. Villeneuve, personal communication). During desynapsis, AIR-2 staining (Fig. 7 C; Fig. S1 E; Nabeshima, K., M. Colaiácovo, and A. Villeneuve, personal communication), like SYP-1 staining (Fig. 6 B, Fig. S1 B; MacQueen et al., 2002), became greatly diminished along the separated regions of each homologue pair but persisted on the synapsed regions of homologues. In contrast, SMC-3 persisted along both the synapsed and desynapsed regions of homologues (Fig. 7 C; Fig. S1 E). A similar pattern of AIR-2 localization was observed in *hcp-6(mr17)* mutants (Fig. 7 C; Fig. S1 E). Because both the localization of AIR-2 between homologues in pachytene and the asymmetric localization to synapsed segments of homologues during pachytene exit are specified before the time of condensin function, AIR-2 localization at the midbivalent (short arm) might also be specified in a condensin-independent manner. In contrast, sister chromatid structure and resolution appeared to be controlled by condensin.

Mutation of *hcp-6* reduces the premature separation of homologues caused by depletion of meiotic cohesin subunit REC-8

If one role of condensin is to achieve proper sister chromatid structure and resolution within diakinesis bivalents, as suggested by the aberrant cohesin staining pattern in condensin mutants, then reducing condensin function should prevent the premature separation of sister chromatids and homologues that results from depleting the meiotic cohesin subunit REC-8 by RNAi. In wild-type animals, sister chromatids are fully resolved by diakinesis, and the cohesin complex holds both sister chromatids (long

Figure 8. Condensin mutations blocked the resolution of cohesin-independent linkages between sister chromatids and between homologues in diplotene–diakinesis. (A) 5S rDNA FISH showed equivalent homologue asynapsis in *hcp-6(mr17);rec-8(RNAi)* double mutants at 15 and 25°C. (B) Both *hcp-6(mr17)* and *mix-1(b285)* blocked precocious homologue and sister chromatid separation caused by *rec-8(RNAi)*. Wild-type diakinesis nuclei contain six bivalents. 7–12 DAPI-staining bodies indicate separation of one or more homologue pairs; more than 12 DAPI-staining bodies indicates separation of both sister chromatids and homologues. *n* = number of oocytes scored. (C) The number and size of DAPI-staining bodies of wild-type, *rec-8(RNAi)*, and *hcp-6(mr17);rec-8(RNAi)* animals showed that *hcp-6(mr17)* prevents premature homologue separation. The arrowhead marks a normal bivalent in a *rec-8(RNAi)* animal. Bars, 5 μ m. (D) The *hcp-6(mr17)* mutation prevents homologue separation in *spo-11(me44)* animals. Similar phenotypes resulted from *spo-11(me44)* at 15 and 25°C ($P = 1$; Table S3, available at <http://www.jcb.org/cgi/content/full/jcb.200408061/DC1>). *n* = number of oocytes scored. (E) Resolution of diakinesis bivalents is delayed in *hcp-6(mr17)* mutants relative to wild type. The percentage of oocytes with any resolved bivalents is plotted against oocyte position in the gonad, which reflects the age of the oocyte (–9 oocyte, early diplotene; –1 oocyte, oldest oocyte). *n* = number of gonad arms scored.



arms of cruciform bivalent) and homologues (short arms of cruciform bivalent) together before segregation in meiosis I and II (Pasierbek et al., 2001; Chan et al., 2003). Without cohesin, sister chromatids and homologues are apart in diakinesis.

Indeed, precocious homologue separation caused by *rec-8(RNAi)* was significantly reduced ($P < 0.01$) by both the *hcp-6(mr17)* and *mix-1(b285)* alleles (Fig. 8, B and C). 100% of wild-type oocytes had six bivalents (six DAPI bodies), but only 4–11% of *rec-8(RNAi)* oocytes grown at either 15 or 25°C had six bivalents (Fig. 8 B). Instead, homologues were often separate in *rec-8(RNAi)* oocytes (7–12 DAPI bodies instead of 6), as were both homologues and sister chromatids (>12 DAPI bodies). In contrast, 68% of oocytes from *hcp-6(mr17);rec-8(RNAi)* animals grown at 25°C had six bivalents, compared with 16% of oocytes from siblings grown at 15°C (Fig. 8 B), demonstrating the reduction in precocious homologue separation by the *hcp-6(mr17)* mutation (Fig. 8 B). Similarly, six bivalents were observed in 35% of oocytes from *mix-1(b285);rec-8(RNAi)* worms, but in only 13% of their *mix-1* heterozy-

gous siblings. The more severe mitotic germline defects in *hcp-6(mr17)* mutants than in *mix-1(b285)* mutants (unpublished data) suggest that *mix-1(b285)* may impair condensin function less than *hcp-6(mr17)*, explaining the smaller effect of *mix-1(b285)* on *rec-8*-deficient gonads (Fig. 8 B). Together, these results indicate that cohesin-independent linkages arise in condensin mutants and hold homologues together, thereby preventing precocious homologue separation in *rec-8(RNAi)* animals. These aberrant linkages likely arise between sister chromatids, allowing homologues to be attached through defective sister chromatid resolution at the short arm of the bivalent.

HCP-6 is required to prevent or to resolve recombination-independent linkages between homologues

We also asked whether any of the *hcp-6(mr17)*-induced linkages had causes other than defective sister chromatid resolution. If so, such linkages might block the precocious separation of homologues that occurs in diplotene–diakinesis when cross-

over recombination is prevented between homologues. Sister chromatid linkages cannot maintain homologue association without recombination. To test this idea, crossover recombination was disrupted in *hcp-6(mr17)* animals by a strong loss-of-function mutation in *spo-11*. The SPO-11 endonuclease makes the double-strand breaks (DSBs) required to initiate meiotic recombination; sister chromatid cohesion is normal in SPO-11 mutants (Dernburg et al., 1998; Pasierbek et al., 2001). Precocious homologue separation was greatly reduced ($P < 0.05$) in *hcp-6(mr17);spo-11(me44)* double mutants raised at 25°C compared with those raised at 15°C (Fig. 8 D, Table S3, available at <http://www.jcb.org/cgi/content/full/jcb.200408061/DC1>): only 15% of oocytes ($n = 158$) from *hcp-6;spo-11* mutants grown at 25°C had 12 univalents compared with 55% ($n = 206$) from those raised at 15°C. Moreover, 11% of oocytes from double mutants grown at 25°C had six DAPI-stained bodies, compared with 0% from those grown at 15°C. Two observations suggested that *spo-11(me44)* blocked recombination. First, *spo-11(me44)* resembles the *bona fide spo-11(ok79)* null allele (Table S3). Second, RAD-51 foci, which mark DSBs destined for recombination, were not apparent on meiotic chromosomes of *hcp-6(mr17);spo-11(me44)* double mutants or *spo-11(me44)* single mutants grown at 15 or 25°C (Fig. S2, available at <http://www.jcb.org/cgi/content/full/jcb.200408061/DC1>), suggesting that DSBs were not present to initiate recombination in *spo-11(me44)* mutants. Thus, our *spo-11* data suggest that one function of condensin is to prevent or to resolve recombination-independent linkages that occur between homologues as they are reorganized around the chiasma in diplotene and diakinesis to form cruciform bivalents.

On a quantitative basis, *hcp-6(mr17)* was markedly more effective at reducing precocious homologue separation caused by *rec-8* RNAi than by *spo-11(me44)* (Fig. 8, B and D). This observation is consistent with the view that *hcp-6(mr17)*–induced defects in either sister chromatid resolution or homologue resolution can contribute to the reduction of *rec-8*-RNAi–induced homologue separation, whereas only aberrant homologue resolution can reduce *spo-11(me44)*–induced homologue separation.

Discussion

In this paper, we show that condensin acts as a chromosome-restructuring complex to drive the transformation of homologues from their extended, parallel arrangement in pachytene nuclei to the compact, cruciform structure observed in diakinesis. After pachytene exit, condensin II subunits HCP-6 and MIX-1 compact desynapsed chromosomes, promote the reorganization of sister chromatids around the crossover, and help prevent or resolve cohesin-independent linkages between sister chromatids and between homologues. These steps are essential for meiotic chromosome segregation, and HCP-6 disruption causes anaphase chromosome bridges during both meiotic divisions.

In a previous analysis of the role of HCP-6 in embryonic mitosis, Stear and Roth (2002) proposed that *hcp-6(mr17)* mutant chromosomes lack the rigidity of wild-type chromosomes; consequently, centromeres twist around the chromosomal axis,

resulting in the merotelic attachment of chromatids to spindle microtubules. We have demonstrated two other mechanisms by which condensin mutation could disrupt chromosome segregation: either the aberrant sister chromatid organization or the persistence of cohesin-independent linkages in *hcp-6* and *mix-1* mutants could promote the anaphase bridges that form between meiotic chromosomes.

Cohesin-independent linkages persist between sister chromatids and between homologous chromosomes in condensin mutants

The removal of protein and DNA-mediated linkages between chromosomes requires the coordinated functions of many enzymes. Because no condensin activity capable of directly resolving chromosomal linkages has yet been described, condensin may promote chromosome resolution through other factors. For example, topo II removes catenation between two DNA strands by breaking one strand and passing the second strand through the break, which it then repairs (Wang, 2002). However, catenation can be introduced by the same mechanism. Decatenation is enhanced by a decrease in local DNA concentration and by pulling forces that aid the separation of decatenated DNA strands (Wang, 2002). Condensin-mediated compaction may promote chromatid resolution both by decreasing the concentration of DNA between sisters and by increasing the tension on catenated strands. We propose that cohesin-independent linkages arise in *hcp-6(mr17)* and *mix-1(b285)* mutants during either the transient decondensation or the recondensation that follows pachytene exit. Loss of condensin function may not only hinder the removal of linkages between chromosomes, but also may actually increase the introduction of de novo linkages.

We have shown that *hcp-6(mr17)* not only results in persistent linkages between sister chromatids, but also between homologues, as demonstrated by the reduced separation of univalent homologues that follows disruption of recombination by a *spo-11* mutation. Synapsis does not require SPO-11 function in *C. elegans* (Dernburg et al., 1998), and synapsed homologues are aligned along their length in wild-type and *hcp-6(mr17);spo-11(me44)* double mutants; perhaps the proximity of homologues in this arrangement permits the formation of linkages.

As in *C. elegans*, the single *S. cerevisiae* condensin complex is required for chromosome segregation in meiosis I and II (Yu and Koshland, 2003). In contrast to *C. elegans*, mutation of yeast *spo11* reduced anaphase chromosome bridging in condensin mutants to nearly wild-type levels, implicating DSB formation and the initiation of recombination in the formation of linkages. Thus, condensin has been proposed to mediate the resolution of recombination-dependent linkages between homologues in budding yeast (Yu and Koshland, 2003). However, budding yeast Spo11 is required for synapsis (Cha et al., 2000), so decreased homologue proximity may also contribute to the reduction of linkages that results from *spo11* disruption in condensin mutants. Because synapsis is diminished by condensin mutations in *S. cerevisiae* (Yu and Koshland, 2003), it is

unlikely that recombination-independent linkages could account for all linkages that form in yeast condensin mutants. Nevertheless, condensin may perform a conserved function that limits the introduction of recombination-independent linkages between homologues during meiotic prophase.

Proteins important for chromosome segregation accumulate on meiotic chromosomes during the formation of cruciform bivalents

The reorganization of chromosomes in diplotene–diakinesis results not only in the resolution of linkages and the reorganization of homologue pairs into compact, cruciform structures, but also in the acquisition of instructional cues that guide chromosome segregation later in meiosis. Micromanipulation experiments, in which meiosis I bivalents were introduced into meiosis II spindles, demonstrated that bivalents acquire the information necessary to direct reductional division before NEBD (Nicklas, 1977; Paliulis and Nicklas, 2000). We propose a molecular explanation for this finding, based on the meiotic localization of proteins important for chromosome segregation. Condensin, centromere protein CENP-A and kinetochore protein HIM-10 colocalize with the four sister chromatids in diakinesis bivalents (Fig. 5 C and unpublished data; Howe et al., 2001). Similarly, AIR-2 accumulates at the mid-bivalent in diakinesis, where it targets cohesin subunit REC-8 for destruction by separase (Kaitna et al., 2002; Rogers et al., 2002). Thus, proteins required for chromosome segregation and for the removal of cohesion that tethers homologues associate with bivalents in prophase, before NEBD.

Transient chromosome decondensation may facilitate the reorganization of meiotic chromosomes

Transient chromosome expansion occurs at the leptotene–zygotene transition in maize (Dawe et al., 1994) and at the pachytene–diplotene transition in many vertebrates and fungi (Zickler and Kleckner, 1999). The requirement for condensin in the reorganization of chromosomes after the leptotene–zygotene transition (*S. cerevisiae*) and after pachytene exit (*C. elegans*) implicates condensin in the large-scale reorganization of chromosomes, which may involve regulated decondensation and recondensation to tear down previous DNA or protein structures in preparation for the creation of new chromosomal architectures.

Materials and methods

Antibodies

Peptide antibodies were produced (see Online supplemental materials and methods, available at <http://www.jcb.org/cgi/content/full/jcb.200408061/DC1>). SYP-1 (MacQueen et al., 2002; from A. Villeneuve, Stanford University School of Medicine, Stanford, CA), AIR-2 (Rogers et al., 2002; from J. Schumacher, University of Texas MD Anderson Cancer Center, Houston, TX), SMC-1 (Chan et al., 2003), and MIX-1 and SMC-4 (Hagstrom et al., 2002) antibodies were described previously.

Biochemistry

Protein analyses were performed as in Chan et al. (2003). MIX-1 IP for microsequencing used 400 µg of affinity-purified antibodies and 480

mg of N2 embryo extract. Coprecipitated proteins were digested with endopeptidase Lys-C (Wako Chemicals) and identified by microsequencing: DPY-28 by KLENEQDAYSRP and KAIQYVFAPDVM, and HCP-6 by KNPENAMDDPD.

Genetics

The following strains were used: wild-type (N2 Bristol), *hcp-6(mr17ts) III* (from M. Roth, University of Washington and Fred Hutchinson Cancer Research Center, Seattle, WA), *unc-4(e120) mix-1(b285) sqt-1(sc13)/mnC1 II*, *hcp-6(mr17ts); unc-119(ed3op) ruls32[unc-119(+)] pie-1::GFP::H2B III*, *spo-1(ok79)/nT1 IV*, *spo-1(me44)/nT1* (from A. Villeneuve).

Homozygous *hcp-6(mr17)* animals were grown at 15°C. Young adult hermaphrodites with a single row of embryos in their uterus were grown at 25°C for 24 h before phenotypic analysis. These conditions reduced meiotic condensin function without causing extensive aneuploidy in the meiotic germline. Embryonic lethality was scored in F1 progeny and germline defects in *unc-4(e120) mix-1(b285) sqt-1(sc13)* homozygotes grown at 20°C and in *hcp-6(mr17)* worms at 25°C.

RNA interference

Templates for dsRNA production are in the Online supplemental materials and methods. Young adult *hcp-6(mr17)* hermaphrodites were injected with 2 mg/ml *hcp-6* RNA, and were then grown at 25°C for 24 h before analysis. CENP-A (4 mg/ml of a 1:1 mixture of dsRNA to *hcp-3* and paralogue F54C8.2) and *mix-1* (3–4.5 mg/ml) RNA were injected into L4 *mix-1(b285)* homozygotes and N2 animals, respectively; gonads and embryos were fixed and stained 36 h later. F1 progeny from N2, *hcp-6(mr17)* homozygotes and *mix-1(b285)* heterozygotes injected with 5 mg/ml *rec-8* were collected as described previously (Chan et al., 2003). Before fixation, sets of 10 progeny (young adults with a single row of embryos in their uterus) from each injected *hcp-6(mr17)* animal were grown for 24 h at 15 or 25°C. Five homozygous and five heterozygous progeny of each injected *mix-1(b285)* heterozygote were processed as described below.

Microscopy

Immunofluorescence and FISH staining were performed and imaged at RT as described previously (Howe et al., 2001). Detergent extraction was used to reduce MIX-1 nucleoplasmic staining: hermaphrodite germlines were dissected in 5.5 µl of sperm salts (Howe et al., 2001) containing 1% Triton X-100. After 3 min, 5.5 µl of 2% PFA (Electron Microscopy Sciences) was added. After 7 min, slides were processed as in Howe et al. (2001). 5S rDNA FISH counts were performed (Chan et al., 2003) on images captured with a camera (C4742-95; Hamamatsu Corporation) mounted on a microscope (Axioplan 2; Carl Zeiss Microimaging, Inc.). SMC-1 and SMC-3 staining were scored in the three oocytes nearest to the spermatheca, and AIR-2 staining in the oocyte nearest the spermatheca. Statistical comparison was calculated by Fisher's exact test (Agresti, 1992). Images were processed with Adobe Photoshop 8.0.

For imaging of meiotic chromosome segregation, animals were prepared as described previously (McCarter et al., 1999). Time-lapse images were obtained in utero using a spinning disc confocal microscope (Perkin-Elmer). Every 30 s, ten 0.75–1-s exposures were acquired at 1-µm intervals with 2 × 2 binning. For publication, image projections were prepared by maximum intensity projection using MetaMorph (Universal Imaging Corp.).

Phylogenetic analysis

Phylogenetic inferences for DPY-28, HCP-6, and known CAP-D2, CAP-D3, and CAP-G homologues were generated by a maximum-likelihood method using ProtML from MOLPHY 2.3 (Adachi and Hasegawa, 1996) and the Jones-Taylor-Thornton model. MUSCLE (Edgar, 2004) and T-COFFEE (Notredame et al., 2000) alignments both found greater relatedness between DPY-28 and CAP-D2 and between HCP-6 and CAP-D3, as corroborated by PSI-BLAST (Altschul et al., 1997) homology predictions. Fig. 1 D is based on the T-COFFEE alignment and rooted to the CAP-G outgroup. The DPY-28 GenBank/EMBL/DBJ accession no. is AAK81894. Other accession nos. are in Ono et al. (2003).

Online supplemental material

The supplemental materials and methods section describes peptides used for antibody production and PCR primer sets used to amplify templates for dsRNA production. Fig. S1 shows an analysis of HCP-6 localization and function in the germline. Fig. S2 shows that RAD-51 foci do not form on meiotic chromosomes of *spo-1(me44)* mutants, suggesting that recombination is blocked. Table S1 shows levels of embryonic lethality in *hcp-6(mr17)* mutants. Table S2 shows that oocyte cellularization occurs relatively normally in *hcp-6(mr17)* mutants. Table S3 shows that HCP-6 is

required to remove or prevent the introduction of recombination-independent linkages between homologues. Online supplemental material available at <http://www.jcb.org/cgi/content/full/jcb.200408061/DC1>.

We thank S. Zhou for peptide microsequencing; A. Villeneuve, J. Schumacher, M. Roth, and A. Gartner for reagents; and A. Chan and C. Tsai for helpful discussion.

R.C. Chan was funded by the Leukemia and Lymphoma Society and the Howard Hughes Medical Institute, A.F. Severson by the Helen Hay Whitney Foundation, and this work by National Institutes of Health grant GM30702 to B.J. Meyer. B.J. Meyer is an investigator of the Howard Hughes Medical Institute.

Submitted: 12 August 2004

Accepted: 5 October 2004

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